25 SCOPOLAMINE AND ATROPINE QUANTITATION AND CONFIRMATION BY LCMS

25.1 Summary

25.1.1 Scopolamine and atropine are extracted from biological samples by making the samples basic with saturated borate buffer and extracting with toluene/hexane/isoamyl alcohol. An aliquot of the extract is analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).

25.2 Specimen Requirements

25.2.1 2 mL blood, fluid or tissue homogenate.

25.3 Reagents and Standards

25.3.1 Atropine, 1 mg/mL

25.3.2 Scopolamine, 1 mg/mL

25.3.3 Pentazocine, 1 mg/mL

25.3.4 Sodium tetraborate decahydrate

25.3.5 Hexane

25.3.6 Isoamyl alcohol

25.3.7 Methanol

25.3.8 Toluene

25.3.9 Acetic Acid

25.3.10 Acetonitrile

25.4 Solutions, Internal Standard, Calibrators and Controls

25.4.1 Saturated borate buffer solution. Add sodium tetraborate decahydrate to dH_2O until no more dissolves after shaking vigorously.

25.4.2 Toluene:Hexane:Isoamyl Alcohol (THIA) (78:20:2, v:v:v) Mix 78 mL toluene, 20 mL hexane and 2 mL isoamyl alcohol.

25.4.3 Drug stock solutions:

25.4.3.1 If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and QS to volume with methanol. Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free drug, and weigh this amount. Stock solutions are stored capped in a refrigerator and are stable for 2 years.

25.4.4 Working standard solution for scopolamine and atropine (SCAT, 0.001 mg/mL): Pipet 10 µL each of the 1 mg/mL stock solution of scopolamine and atropine into a 10 mL volumetric flask and QS to volume with methanol.

25.4.5 Working internal standard solution (0.001 mg/mL pentazocine): Pipet 10 µL of the 1 mg/mL stock solution of pentazocine into a 10 mL volumetric flask and QS to volume with methanol.

25.4.6 To prepare the calibration curve, pipet the following volumes of the 0.001 mg/mL SCAT working solution into appropriately labeled 16 x 125 mm screw cap test tubes. Add 2 mL blank blood to obtain the final concentrations listed below.

Amount of 0.001 mg/mL SCAT working solution (µL)	Final concentration of SCAT (µg/L)
200	100
100	50
40	20
20	10
10	5
4	2
2	1

25.4.7 Controls

10.1.1.1 SCAT Controls. Control may be from a external source or prepared in house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

25.4.7.1 Negative control. Blood bank blood or equivalent determined not to contain scopolamine, atropine or pentazocine.

25.5 Apparatus

25.5.1 Test tubes, 16 x 125 mm, round bottom, borosilicate glass with Teflon caps

25.5.2 Test tubes, 16 x 114 mm, glass centrifuge, conical bottom

25.5.3 Centrifuge capable of 2000-3000 rpm

25.5.4 Nitrogen evaporator with heating block

25.5.5 Vortex mixer

25.5.6 GC autosampler vials with inserts

25.5.7 LC/MS: Agilent Model 1100 LC-MSD

25.5.7.1 LCMS Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

25.5.7.1.1 Elution Condicitons

25.5.7.1.1.1 Column: Agilent Hypersil BDS 125 mm X 3 mm, 3 µM particle size
25.5.7.1.1.2 Column thermostat: 35° C
25.5.7.1.1.3 Solvent A: 45% water with 1% acetic acid
25.5.7.1.1.4 Solvent B: 55% methanol

25.5.7.1.1.5 Isocratic elution, stop time: 6.20 min

25.5.7.1.2 Spray Chamber

25.5.7.1.2.1 Ionization Mode: Electrospray
25.5.7.1.2.2 Gas Temperature: 350° C
25.5.7.1.2.3 Drying Gas (N_2): 12.0 L/min
25.5.7.1.2.4 Nebulizer pressure: 30 psig
25.5.7.1.2.5 Vcap (Positive): 4000 V

25.5.7.1.3 Selected Ion Monitoring (quantitation ions)

25.5.7.1.3.1 Polarity: Positive
25.5.7.1.3.2 Injection volume: 8 µL

Time (min)	Group Name	SIM Ion	Frag-Mentor	Gain EMV	SIM Resol.	Actual Dwell
0.0	Scopolamine	138	220	1.0	Low	195
		156			195	
		304			195	
0.0	Atropine	93	220	2.0	Low	195
		124			195	
		290			195	
4.2	Pentazocine	173	220	1.0	Low	392
		218	250		392	
		286	250		392	

25.6 Procedure

25.6.1 Label clean 16 x 125 mm screw cap tubes appropriately with calibrators, controls and case sample IDs.

25.6.2 Prepare calibrators and controls.

25.6.3 Add 2 mL case specimens to the appropriately labeled tubes.

25.6.4 Add 100 µL 0.001 mg/mL pentazocine internal standard working solution to each tube for a final concentration of 2mg/L.

25.6.5 Add 2 mL saturated borate buffer and 6 mL extract solvent (78:20:2 THIA) to each tube.

25.6.6 Cap and rotate tubes for 30 minutes.

25.6.7 Centrifuge at approx 2500 rpm for 15 minutes. Transfer organic upper layer (THIA) to appropriately labeled conical bottom test tubes.

25.6.8 Evaporate samples to dryness at approximately 50° C under nitrogen.

25.6.9 Reconstitute samples in 500 µL acetonitrile.

25.6.10 Add 1 mL hexane to each tube. Vortex each sample for 30 seconds.

25.6.11 Centrifuge at approx 2500 rpm for 10 minutes. Aspirate and discard upper hexane layer.

25.6.12 Evaporate samples to dryness at approximately 50° C under nitrogen.

25.6.13 Reconstitute samples in 100 µL methanol. Transfer to GC autosampler vials for analysis by LCMS.

25.7 Calculation

25.7.1 Drug concentrations are calculated by linear regression analysis using the ChemStation software.

25.8 Quality Control and Reporting

25.8.1 See Toxicology Quality Guidelines

25.9 REFERENCES

25.9.1 J Saady and A Poklis. Determination of Atropine in Blood by GCMS. J Anal Tox 13: 296-299, 2001.

25.9.2 J Pearson and R Steiner, in-house development.